Wing morphology and eyespot pattern of *Erebia medusa* (Lepidoptera, Nymphalidae) vary along an elevation gradient in the Carpathian Mountains

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Abstract. Butterfly wings play a crucial role during flight, but also in thermoregulation, intraspecific signalling and interaction with predators, all of which vary across different habitat types and may be reflected in wing morphology or colour pattern. We focused on the morphological variability of *Erebia medusa* in order to examine patterns and variations in the colouration and morphology of wings from areas representing different habitat types with different environmental characteristics. The barrier (larger fragments of forest) between populations of *Erebia medusa* along the elevation gradient of Kojšovská hoľa might be the aspect that hinders the movement of the population. The wing characteristics (shape, size, spotting) of males representing populations of Carpathian mountain habitats (Volovské vrchy, Ondavská vrchovina) located at different elevations were measured. The forewing shape analysis, using geometric morphometry based on 16 landmarks, showed significant differences between populations from different elevation levels. The pattern of the forewings also varied between populations. Morphological changes among individuals of *Erebia medusa* populations along the elevation gradient in the Carpathian Mountains showed that in the cold, highland habitats we observed smaller, narrower and elongated forewings with a reduced number of spots, while males from warmer habitats at low elevations had rounder, larger and more spotted forewings.

Introduction

The ecological role of individual butterfly species is largely reflected in the wings, whose shape, size and colour pattern often have adaptive value and provide information about important differences, even at the population level (Altizer and Davis 2010; Mega 2014). The variability of butterfly wing shape or size, which reflects flight performance (Cespedes et al. 2015; Le Roy et al. 2019a, b), can even provide insight into the suitability of the habitat (Pellegroms et al. 2009; Chazot et al. 2016) and the dispersal rate (Wells et al. 2018; Taylor-Cox et al. 2020). The final wing shape and size of adults depends on conditions of larval development, which can be affected by aggregation behaviour (Allen 2010; Montejo-Kovacevich et al. 2019; Palmer et al. 2019) but also by environmental conditions (Karl and Fischer 2008; Gibbs et al. 2011; Van Dyck et al. 2016; Palmer et al. 2019). Phenotypic clines along environmental gradients can sometimes be explained

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by ecological rules, whose use on insects can be debatable (Blanckenhorn and Demont 2004). Bergmann's rule is the classic ecogeographic principle that relates the body size of endotherms with environmental temperature (or latitude) (Shelomi 2012). The converse of Bergmann's rule (Park 1949; Mousseau 1997), based on the season length effect, predicts a decrease of body size with elevation. Various clines in body size can also be explained by a combination of several other theories or hypotheses, such as the north-south cline theory (Nylin and Svärd 1991) or the "temperature – size rule" (Angilletta and Dunham 2003).

The wing eyespot pattern, which may serve different functions, can also play an irreplaceable role. While the pattern on the dorsal side is usually used for intraspecific communication (Oliver et al. 2009; Westerman et al. 2012; Tokita et al. 2013), the eyespots on the ventral side are rather used to deceive predators by intimidation or deflection by distracting predators from the vital, vulnerable body parts (Lyytinen et al. 2003; Stevens 2005; Stevens et al. 2007; Kodandaramaiah 2011; Prudic et al. 2015; Ho et al. 2016). Moreover, in several butterfly species, wing colour modifications are related to thermoregulation (Dennis and Shreeve 1989; Taylor-Cox et al. 2020).

Previous studies (Nice et al. 2005; Jugovic et al. 2018) have demonstrated that populations separated by time, space or geographical barrier may undergo changes in the shape, size and colouration of external traits (Tatarinov and Kulakova 2013). Restrictions of the movement and migration of butterfly species have an impact on the intensity and direction of gene flow between populations (Andrews 2010; Slatkin and Excoffier 2012). Characterisation of the morphological traits of *E. medusa*, a species inhabiting a wide range of environments, can provide insight into the selection pressures that affect adaptive responses (Cespedes et al. 2015; Taylor-Cox et al. 2020).

For the sedentary butterfly *Erebia medusa*, high intraspecific variability (numerous subspecies) and mosaic distribution throughout most of its Euro-Siberian region is characteristic (Warren 1936; Schmitt et al. 2000; Polic et al. 2014). Our study focused especially on the influence of elevation differences in the Carpathian region on intraspecific variation. For this species, large fragments of forests (Schmitt et al. 2000) may be a serious obstacle for movement. According to the study by Kleckova and Klecka (2016), *E. medusa* prefers a warm environment, so the adaptations to high elevation habitats needed for the activity of this species can be expected. Lower activity due to low temperature can cause a decrease of chances of escape; therefore, selection will act against some individuals (large sized or with large eyespots) (Dennis et al. 1986). A higher number of eyespots, which are important especially in escape mechanisms, may reflect increased rates of predation with rising temperature (Hillebrand et al. 2009; Vucic-Pestic et al. 2011) but also by sexual selection (Tokita et al. 2013). Based on morphological features (wing size, shape, colour pattern) examined by traditional and geometric morphometry, we focused on the morphological differences between populations from habitats differing in elevation and separated by forest areas.

We predicted that the morphological diversity between *E. medusa* populations would show changes that correlate with the average annual temperature, which varies within the elevation gradient. Our study is based on the hypothesis that i) morphological traits of males (size, shape and pattern of forewings) vary in response to various environmental conditions within an elevation gradient. We also focused on examining whether ii) the forewing size of individuals from higher elevations is smaller than the forewing size of individuals from lower and warmer regions, which induce longer feeding periods during larval development (Juhász et al. 2016). Further, iii) males from higher elevation habitats with lower temperatures were expected to have aerodynamically (narrower, angular) shaped wings that reduce energy costs (Dudley

2002; Lentink et al. 2007; Kovac et al. 2012). Finally, iv) a reduction in the eyespot number with elevation, involving various selection pressures, was expected (Slabý 1950; Tatarinov and Kulakova 2013).

Materials and methods

Studied species

The studied species, the Woodland Ringlet – *Erebia medusa* (Denis & Schiffermüller, 1775) (Lepidoptera, Nymphalidae: Satyrinae) – inhabits areas from lowlands to sub-alpine zones (Sonderegger 2005) within a wide elevation range (Warren 1936). This species prefers mesophilous habitats, including forest-steppes, pastures, forest meadows, mesophilous meadows and peat bogs with a transitional character, but also calcareous grasslands. The presence of this species within the (Euro-Siberian) distribution range has been recorded from central France, across Central and Eastern Europe (including the Balkans), southern Siberia, Mongolia, northern China to eastern Asia. Adults, especially in the western parts of the distribution, occur from late April to June, and in highlands can be observed to July or in some areas to early August. Females of this univoltine species lay eggs on the tops of foodplant stalks, represented by grasses, such as *Nardus* spp., *Festuca* spp. (e.g., *Festuca ovina*) and *Bromus* spp. (e.g., *Bromus erectus*). Depending on the habitat conditions, the solitary caterpillars hibernate once or twice (at high elevations) (Settele et al. 2008).

Field sampling

One hundred males of *E. medusa* from localities in the Volovské vrchy Mts. (Kojšovská hoľa – KH 1246 m a. s. l, Zlatá Idka – ZL 660 m a. s. l) and from the Ondavská vrchovina Mts. (Dobroslava – DB 335 m a. s. l) were analysed (Fig. 1). The morphological differences in the populations were studied along an elevation gradient (See Suppl. material 1: Satellite view) between Kojšovská hoľa, Zlatá Idka and Dobroslava. Kojšovská hoľa represents a high elevation habitat with low average temperatures (T_{KH} = 5.7 °C); Zlatá Idka and Dobroslava, with higher average temperatures (T_{ZL} = 7.4 °C; T_{DB} = 8.9 °C), are located at relatively low elevations (see Suppl. material 2: Characteristics of localities). Habitats of the Volovské vrchy Mts. (Kojšovská hoľa and Zlatá Idka) are separated by large fragments of forest. The butterflies were captured from May to July during the years 2018–2020 using a net, and each location was visited six times per month. All specimens were dried and pinned; the wings were detached using forceps (Paučulová et al. 2018) and stored separately in plastic bags.

Analysis of wing pattern and morphology

The front wings of each individual were photographed under standardised light conditions from the dorsal side using an Olympus digital camera MODEL NO. C-5060 white zoom DC GV connected to an Olympus SZ2-ILST stereomicroscope at 6.4× magnification using the program QuickPhoto MICRO 2.1 (Fig. 2)

Male forewing length and width across localities were compared using traditional morphometric methods (Marcus 1990; Dapporto 2008). Wing shape was analysed using geometric morphometric methods (Prieto et al. 2009; Chazot et al. 2016) in the MorphoJ program (Fig. 2A). The geometric morphometric analysis of the forewing was based on 16 landmarks situated at the vein intersections and vein terminations (Fig. 2B) (Benítez et al. 2011) to ensure the repeatability of each landmark in the highest number of individuals (Habel et al. 2011). The dataset with the exam-

Figure 1. Map of sampling sites. **1.** Kojšovská hoľa (1246 m a. s. l); **2.** Zlatá Idka (660 m a. s. l); **3.** Dobroslava (335 m a. s. l).

ined traits was converted to a tps-file using the program tpsUtil, and landmarks were set with the program tpsDig2 Version 2.16. Landmarks were set on each individual twice in order to minimise measurement errors (Arnqvist and Martensson 1998). To analyse the impact of environment we used data on the mean annual air temperature. Information about the temperature conditions at each site was monitored using dataloggers (Thermochron iButton device DS1921G) placed on a tree trunk (sampling frequency every 4 hours/over a year).

Statistical analyses

The obtained data (untransformed data) was evaluated using the statistical parameters of mean (M), standard deviation (SD) and coefficient of variation (CV). The normal distribution of forewing length, width and shape data, separated in terms of side (L, R), was confirmed by normality tests (the Shapiro-Wilk W test, the Anderson-Darling A test, the Jarque-Bera JB test) using PAST version 3.11 (Hammer et al. 2001); therefore, raw data were used in the subsequent analyses. Data on wing colour pattern were tested for normality using the Shapiro-Wilk W test.

Differences in the morphological traits (wing width, wing length) among the sites were assessed using ANOVA and Tukey's pairwise comparisons implemented in PAST version 3.19 (Hammer et al. 2001). To estimate the relationship between environmental variables and forewings, we used a multiple linear regression model, using forewing length and width as dependent variables and elevation and temperature as independent variables. Statistical analyses were performed using PAST 3 (Hammer et al. 2001).

The shape of the forewings was compared among the populations following the standard procedure consisting of the location of landmarks (at the costal margin and the nodes of veins), principal component analysis (PCA) based on the covariance matrix, canonical variate analysis (CVA) and multivariate analysis (ANOVA, Kruskal-Wallis test). ANOVA and CVA were used to compute morphological variabilities between the left and right forewing side.

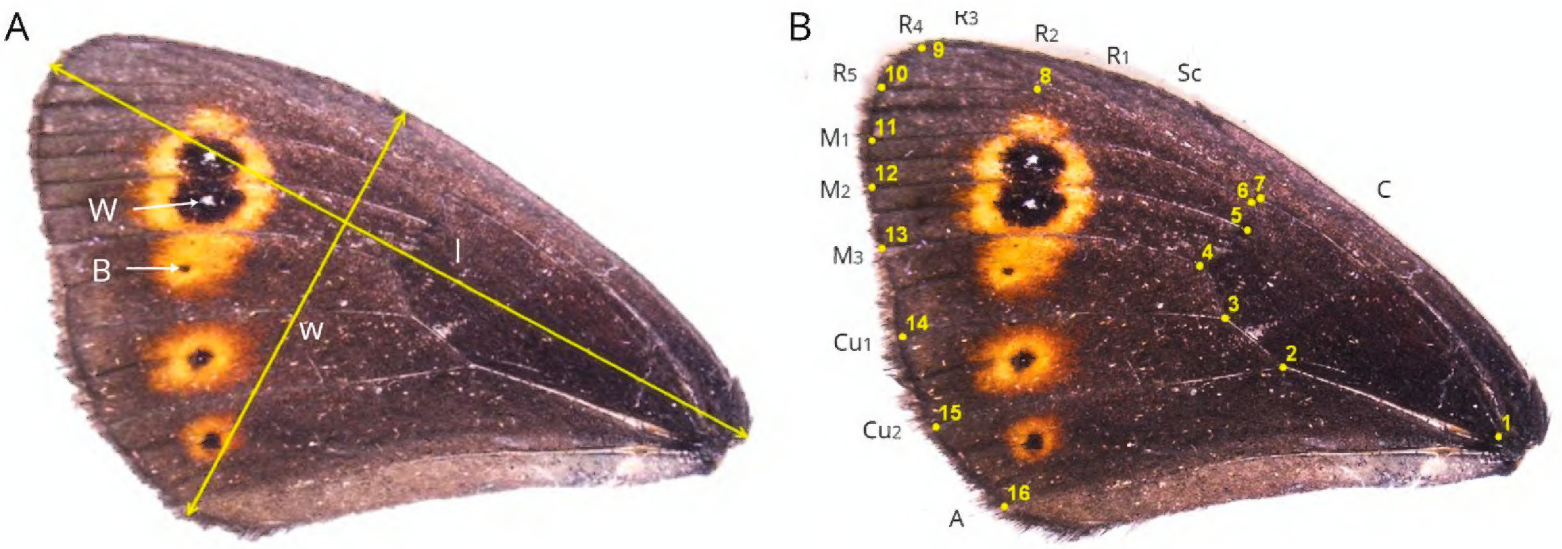

Figure 2. The left forewing of an *Erebia medusa* male used for (**A**) traditional and (**B**) geometric morphometrics. **A.** Studied traits. W – width, l – length, B – black pattern, W – white pattern. **B.** Distribution of landmarks (LM 1–16). Landmarks: 1 – intersection of veins at the base of the discal cell, 2 – Discal cell and Cu2 intersection, 3 – Discal cell and Cu1 intersection, 4 – Discal cell and M3 intersection, 5 –Discal cell and M2 intersection, 6 – Discal cell and M1 intersection, 7 – Discal cell and R4 Intersection, 8 – R4 and R5 intersection, 9 – R4 terminally, 10 – R5 terminally, 11 – M1 terminally, 12 – M2 terminally, 13 – M3 terminally, 14 – Cu1 terminally, 15 – Cu2 terminally, 16 – A terminally.

The forewing shape variation among populations in the dataset were analysed using PCA. The results of CVA, which assessed the inter-location differences, were reported as respective *P* values for the Procrustes distances, after permutation tests (10,000 runs). The shape variation among localities was additionally analysed using ANOVA and post hoc tests. The mentioned statistical and geometric morphometric analyses were performed in MorphoJ (Klingenberg 2011), a program for geometric morphometrics.

We further analysed pattern elements from digital images and examined the presence and number of white and black spots on the dorsal side of the forewing margin. We examined the difference in the number of spots between populations in the program PAST version 3.1. The non-parametric Kruskal-Wallis test was performed to test the statistical significance of differences, and post hoc tests were performed to compare the samples. The mean and standard deviation (SD) values were calculated. Possible correlation between variables was examined using Spearman's rank correlation coefficient (ρ). The program MorphoJ version 1.01 (Klingenberg 2011) was used to compute morphological variabilities among populations, among individuals and between the left and right wing-side of one butterfly individual, as well as measurement errors in the wing shapes.

Results

The intraspecific variability of wing morphology among the 100 samples (males) was analysed. All individuals were checked for measurement and digitisation errors, which were minimised (Štefánik and Fedor 2020). The results of ANOVA revealed statistically significant differences in wing length and width (length: $F(2, 97) = 17.38$, $p < 0.0001$, width: $F(2, 97) = 34.19$, $p < 0.0001$) between all sites (length and width: Tukey's post hoc test, Kojšovská hoľa (1246 m a. s. l) vs Dobroslava (335 m a. s. l), $p < 0.001$; Kojšovská hoľa (1246 m a. s. l) vs Zlatá Idka (660 m a. s. l) $p < 0.001$, with the exception

of Dobroslava vs Zlatá Idka, which did not show significant differences in the length or width of the forewings ($p = 0.702$). The measurements of the left and right male forewings for each population did not differ significantly (width: T-test $p > 0.05$; length: T-test $p > 0.05$). A multiple linear regression analysis was performed to evaluate the effect of elevation and temperature on the forewings. Our results from the multiple linear regression analysis (length: SS = 63.41; $F(2, 97) = 16.69$; $p < 0.0001$; width: SS = 44.29; $F(2, 97) = 33.13$; $p < 0.0001$) showed a positive relationship of forewing length and width with the mean annual air temperature ($T_{KH} = 5.7$ °C; $T_{ZL} = 7.4$°C; $T_{DB} = 8.9$ °C) (Table 1) but not with elevation. The results indicate that the length and width of male forewings increased with habitat temperature (summary statistics: $\mu \pm SD$; KH: length $\mu = 21.73 \pm 1.34$, width $\mu = 12.26 \pm 0.8$; ZL: length $\mu = 23.29 \pm 1.01$ width, $\mu = 13.66 \pm 0.64$; DB: length $\mu = 23.55 \pm 1.61$, width $\mu = 13.75 \pm 0.94$) (Fig. 3A, B). In contrast, the length/width ratio increased with elevation (Fig. 3C).

Table 1. Results of multiple linear regression on variables wing width and length, with temperature and elevation as fixed factors.

Dependent variable	Fixed effect	Coefficient	SE	t	df	p
FW length	(intercept)	21.194	1.611	13.154	1	1.22×10^{-78}
	temperature	1.310	0.333	2.172	1	0.032
	elevation	-0.262	0.603	-0.786	1	0.434
FW width	(intercept)	11.225	0.956	11.745	1	2.59×10^{-79}
	temperature	1.308	0.358	3.655	1	4.1×10^{-4}
	elevation	-0.090	0.197	-0.458	1	0.648

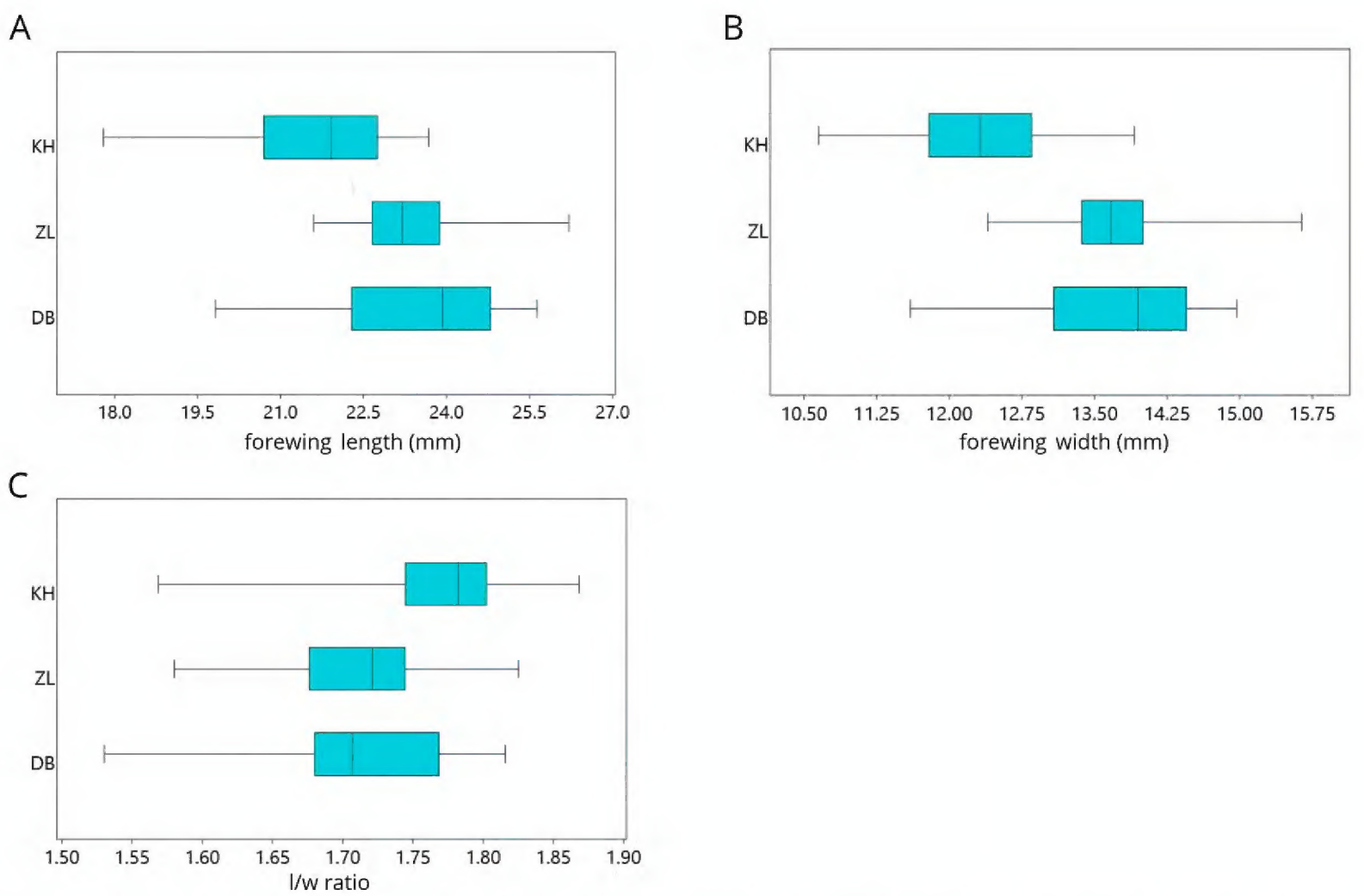

Figure 3. Comparison of *Erebia medusa* forewing length (**A**), width (**B**), length/width ratio (**C**) among the studied populations: KH – Kojšovská hoľa (1246 m a. s. l.); ZL – Zlatá Idka (660 m a. s. l.), DB – Dobroslava (335 m a. s. l). Data are shown as boxplots with minimum, median and maximum values and 25% and 75% percentiles.

The variation of forewing shape between populations was supported by ANOVA (F = 6.71, df = 56, $p < 0.0001$) and further identified by principal component analysis (PCA) applied on the forewing landmarks. Changes in symmetry between the left and right forewings were not significant (ANOVA: $F = 3.64$, df = 28, $p = 0.075$). PCA revealed shape differences in the male forewings between the high elevation locality (Kojšovská hoľa, 1246 m a. s. l) and the low elevation sites. PC1 accounted for 29.3% and PC2 for 18.2% of the detected variation between populations. Canonical variate analysis (CVA) carried out with three groups along the first two canonical variate axes (CV1 and CV2) (Fig. 4) differentiated the KH population from the other two populations along CV1 (70% of the total variation). The ZL and DB populations were partially differentiated along the CV2 (30%) axis. The changes were detected in the area of the disk cell and the intersection with veins M1, M2 (lm 1,6,7), in the apical area (lm 9,10) and the total margin of the forewing (lm 15–16). The results indicate that the forewing shape of males inhabiting the cold, high elevation site (Kojšovská hoľa) was more angular, elongated and narrower than male forewings from warmer sites at lower elevations (Zlatá Idka, Dobroslava). A significant shift and prolongation of the flight period was detected (in highlands (KH, 1246 m a. s. l): from the middle of June to early July – 18 days; in the lower area (ZL, 660 m a. s. l): from the end of May to the middle of June – 22 days). The flight periods of males started earlier and also reached maximum abundance earlier than in females (Suppl. material 3).

The forewing eyespots of male specimens from high elevation populations on Kojšovská hoľa (1246 m a. s. l.) had on average fewer white ($\mu = 2.95$) and black spots ($\mu = 4.16$) than males in the low elevation populations of Zlatá Idka (660 m a. s. l.) and Dobroslava (335 m a. s. l) (white spots: $\mu = 3.72$, black spots: $\mu = 4.61$), with a median difference of 1 for both white and black spots. In summary, with increasing elevation and decreasing temperature males tend to have fewer eyespots on average. Moreover, the overall number of white spots is lower than the number of black spots on the forewings.

The number of black spots on the dorsal side of the male forewing significantly differs between populations (Kruskal-Wallis test: $p = 0.046$), with Dunn's post hoc test showing the biggest difference between populations KH and ZL ($p = 0.013$). The white colour pattern in the centre of the eyespots was also significantly different between the populations (Kruskal-Wallis test $p = 0.012$). The exact differences in the number of white spots between the populations KH (1246 m a. s. l.) vs ZL (660 m a. s. l) ($p = 0.015$) and KH (1246 m a. s. l.) vs DB (335 m a. s. l) ($p = 0.006$) were detected using Dunn's post hoc test. The frequencies also varied depending on the location (Figs 5, 7). Moreover, the overall difference between the number of black (Fig. 7) and white spots (Fig. 8) was confirmed with the Kruskal-Wallis test ($p < 0.0001$; Dunn's post hoc tests KH: $p < 0.0001$; ZL: $p < 0.001$; DB: $p < 0.001$). A statistically significant correlation between black and white spots was examined using Spearman's rank correlation coefficient (ρ) ($\rho = 0.69$; $p < 0.0001$; KH: $\rho = 0.59$; $p < 0.001$, ZL: $\rho = 0.77$; $p < 0.0001$; DB: $\rho = 0.65$; $p < 0.0001$).

Discussion

The morphology of *Erebia medusa* male forewings studied by methods of traditional and geometric morphometrics showed statistically significant differences between populations from low and high elevations differing in annual average temperatures. We found that larger butterflies were generally found in warmer habitats. Smaller and narrower shaped forewings with a fewer number of spots on the dorsal surface were detected in the cold habitat – Kojšovská hoľa (1246 m a. s. l.). The results of our study are consistent with previous findings of the impact of elevation

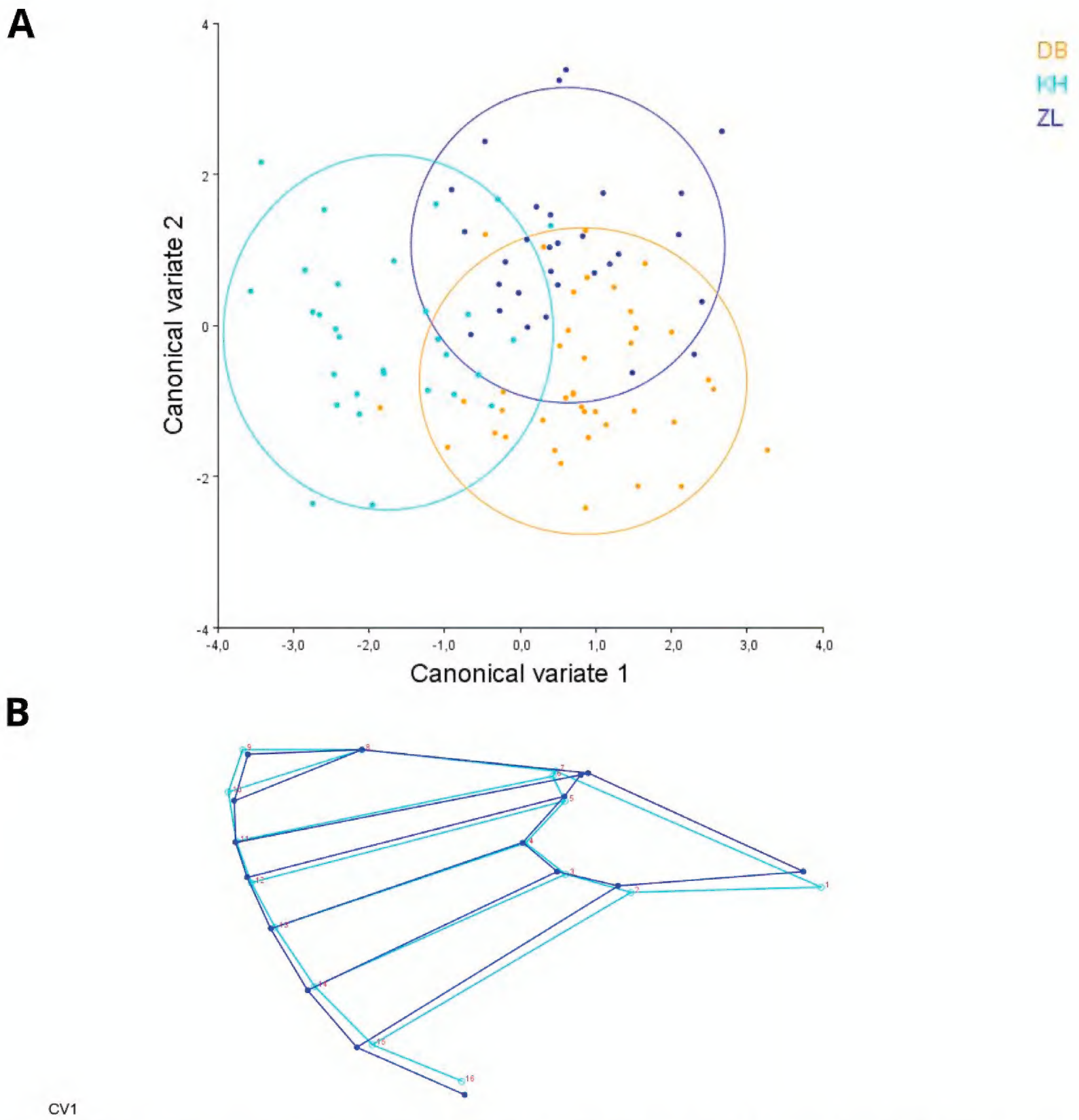

Figure 4. CVA results: **A.** the variation in the forewing shape of studied populations along the first two canonical variate (CV1 and CV2) axes with 95% confidence ellipses of means for each group. Kojšovská hoľa (KH), Zlatá Idka (ZL), Dobroslava (DB); **B.** wireframe graph of the morphometric overlap in forewing shape according to CV1 show deformation (dark blue line) from consensus configuration (sky blue line) associated to each canonical axis.

(Blanckenhorn and Demont 2004) and temperature (De Jong et al. 2010; Bowden et al. 2015) on the variability of morphological traits. Changes in forewing traits among populations in habitats with different climatic conditions may indicate their importance for adaptation to varying intensities of environmental factors.

According to the clinal variation detected as size differences between populations, our results are in line with the season-length effect described by the converse Bergmann's rule, which

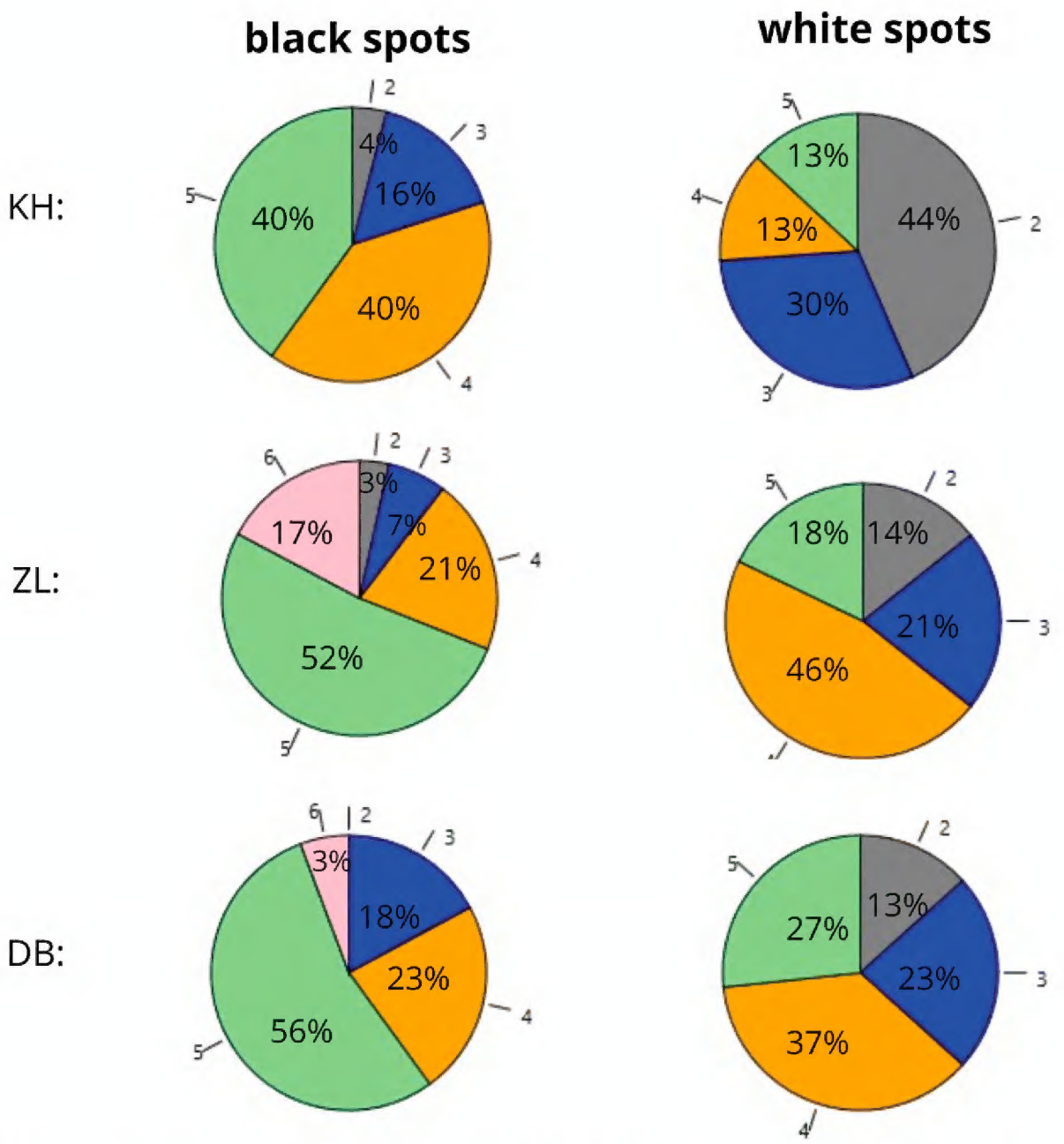

Figure 5. The frequencies of black and white spots in the wing pattern of *Erebia medusa* from three studied habitats. KH – Kojšovská hoľa (1246 m a. s. l), ZL – Zlatá Idka (660 m a. s. l), DB – Dobroslava (335 m a. s. l).

states that depending on the season length, larger individuals can be found at lower elevations (Park 1949; Mousseau 1997). Our findings are consistent with the theory that the smaller size of butterflies at cold, high elevations is a response to decreased time available to grow and survive to the adulthood stage (Rowe and Ludwig 1991). A study by Kleckova and Klecka (2016) showed that lowlands provide optimal conditions for *E. medusa*, which may also contribute to the explanation of bigger individuals from warmer habitats observed in the area of Zlatá Idka and the Dobroslava locality. Our findings indicate that *Erebia medusa* has a similar strategy to avoid developmental constraints in colder environmental conditions, which are suboptimal for development, as in the case of *Dryas iulia* (Fabricius, 1775) (Mega 2014), when lower temperatures and low host-plant availability or quality (Pellegroms et al. 2009; Talloen et al. 2009) is reflected in the smaller body size of butterflies. The longer and wider forewings of males from warmer areas can be explained by the longer availability of food plants, which enable the larvae to grow larger, as previously shown by Bowden et al. (2015) in the arctic butterfly species *Boloria chariclea* (Schneider, 1794) and *Colias hecla* (Lefèbvre, 1836), but also Juhász et al. (2016) in *Melitaea athalia* (Rottemburg, 1775).

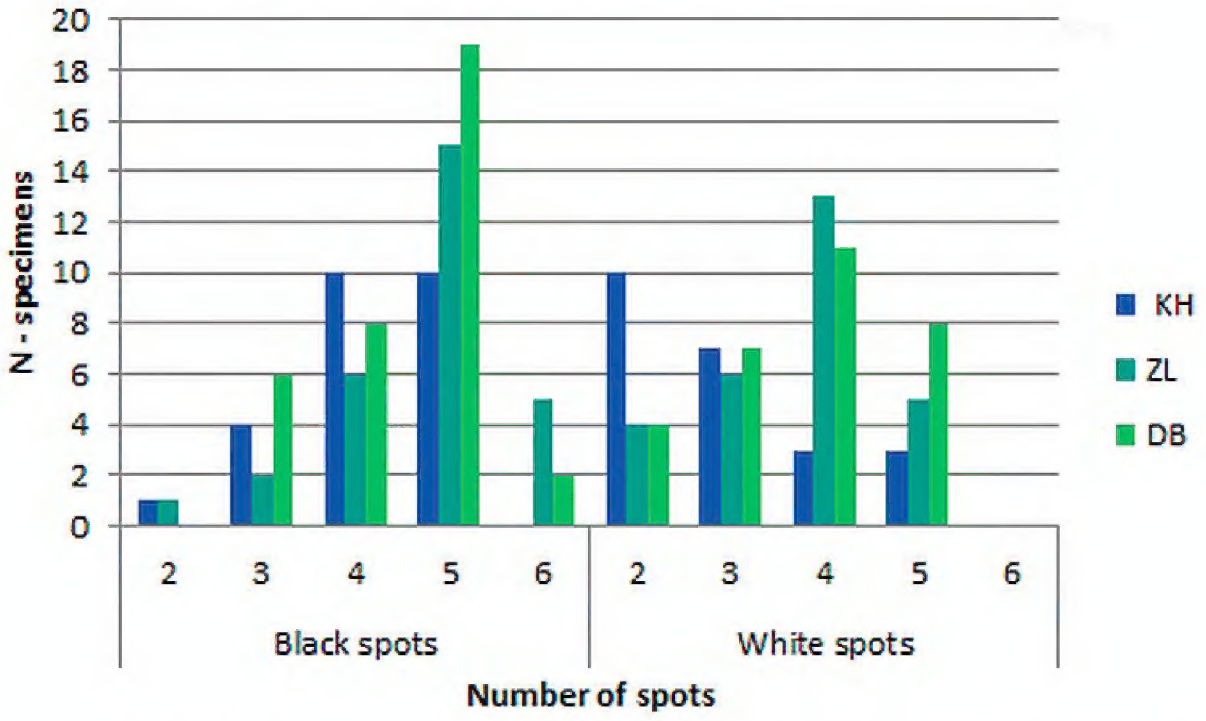

Figure 6. Graph of forewing pattern (dorsal surface) in *E. medusa* populations. KH – Kojšovská hoľa (1246 m a. s. l), ZL – Zlatá Idka (660 m a. s. l), DB – Dobroslava (335 m a. s. l).

Results from geometric morphometrics demonstrate that the forewing shape of males inhabiting the cold, high elevation site (Kojšovská hoľa) was more angular, narrower and slightly elongated, while rounder wings were detected at low elevations. The obtained results are similar to the conclusions on wing shape variance of *Speyeria diana* (Wells et al. 2018) (Cramer and Stoll 1777) resulting from elevation differences. The morphological changes may be explained by various behavioural, functional or physiological mechanisms. From the functional aspect, changes of wing shape play a key role in flight aerodynamics (Park et al. 2010) and may enhance manoeuvrability, as shown by Dudley (2002), Shreeve et al. (2009), DeVries et al. (2010) and Cespedes et al. (2015). The rounder shape was shown to be effective when patrolling and to allow better escape from predators. Elongation of the wings at the Kojšovská hoľa locality may even be the result of poor nutrition of caterpillars, as shown in *Paragre aegeria* (Linnaeus, 1758) (Pellegroms et al. 2009). Elongated wings *E. medusa* were observed in migrating populations (Dudley and DeVries 1990) and in patrolling males (DeVries et al. 2010), probably due to the long endurance flight benefits (Dudley 2002; Dockx 2007; Altizer and Davis 2010). Therefore, our findings of elongation and changes of *E. medusa* wing shape are in accordance with the assumption that such changes, associated with more efficient gliding flight in windy high elevation areas, are required to minimise flight costs (Klok and Harrison 2009; DeVries et al. 2010; Cespedes et al. 2015; Wels et al. 2018; Le Roy et al. 2019a). The wing shape of males varies with elevation (Kojšovská hoľa vs Zlatá Idka and Dobroslava), which corresponds with the findings of Klok and Harrison (2009) or Wells et al. (2018), but also with latitude (Kojšovská hoľa and Zlatá Idka vs Dobroslava), which is consistent with the results of Sanzana et al. (2013) and Taylor-Cox et al. (2020). The shape variability of butterfly forewings exposed to the windy and cold conditions of the Kojšovská hoľa highlands may stem from the need to adapt to more demanding flight conditions, similarly as shown in research on insect flight performance by Combes and Dudley (2009). Our results are in accordance with the research of Le Roy et al. (2019a) and Chazot et al. (2016), presuming that sex-specific behaviour (Cespedes et al. 2015), habitat and interactions with predators are the main selective forces that contribute to butterfly wing shape and pattern evolution.

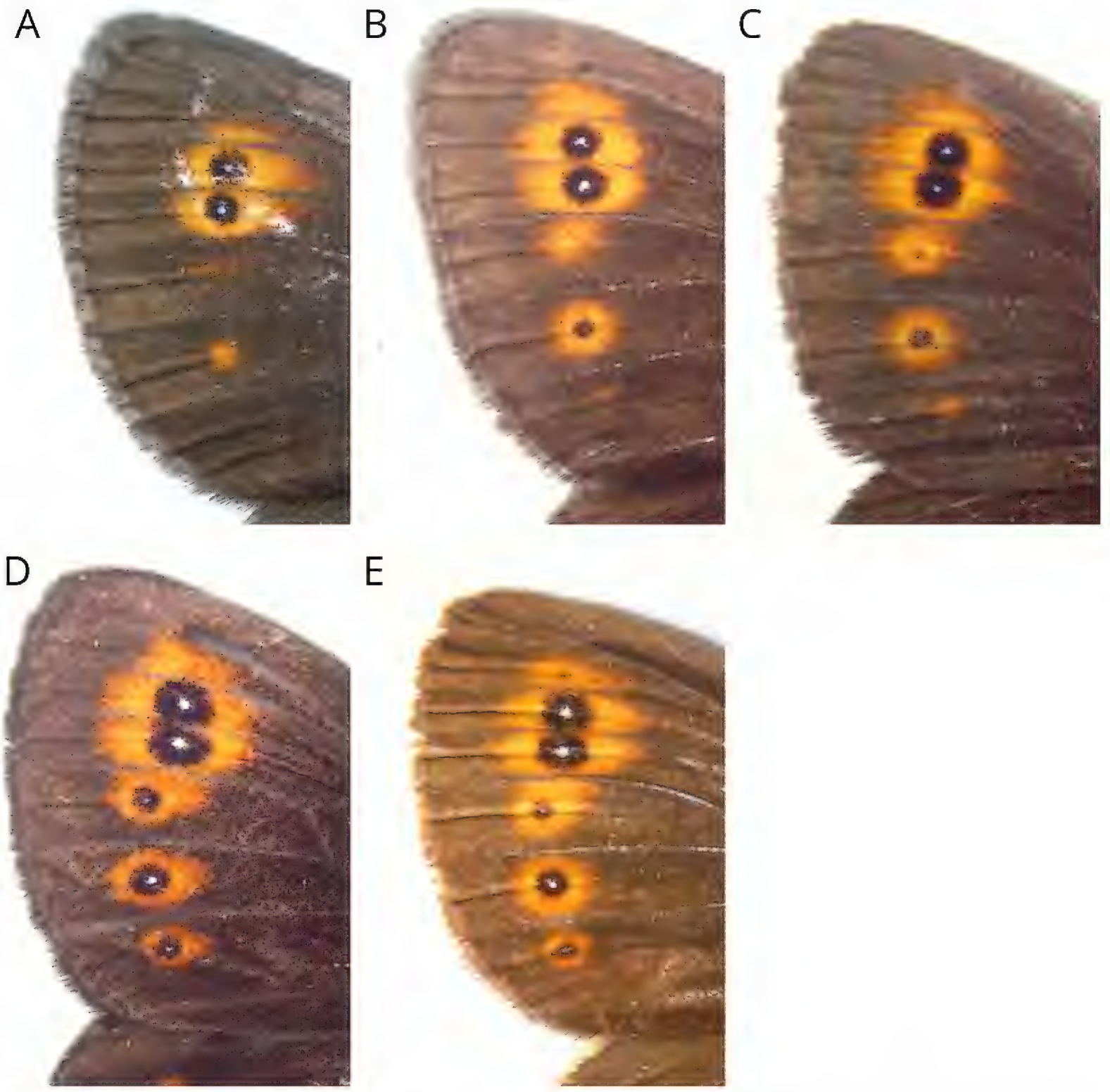

Figure 7. The different pattern of male forewings depending on the number of black spots in the pattern: **A.** 2, **B.** 3, **C.** 4, **D.** 5, **E.** 6.

Our findings of smaller male *E. medusa* in the high and cold environments of Kojšovská hoľa and larger males originating in the lowlands are in line with theory, which underlines the thermoregulatory function of such adjustments in the size and colour of the wings. However, our results on the number of eyespots are partly consistent with the findings of Cassel-Lundhagen et al. (2020) in *Coenonympha arcania*; thus, it seems that size is often related to abiotic factors, while colour patterns and spots on wings (predator escape) may be less associated with environmental constraints.

In addition, the average number of eyespots on male forewings increased with average temperature and decreased with temperature and rising elevation. Our findings confirmed the observations from previous research on *Erebia medusa* in eastern Slovakia conducted by Slabý (1950). A similar reduction of the forewing eyespot pattern in the colder conditions of northern areas was also

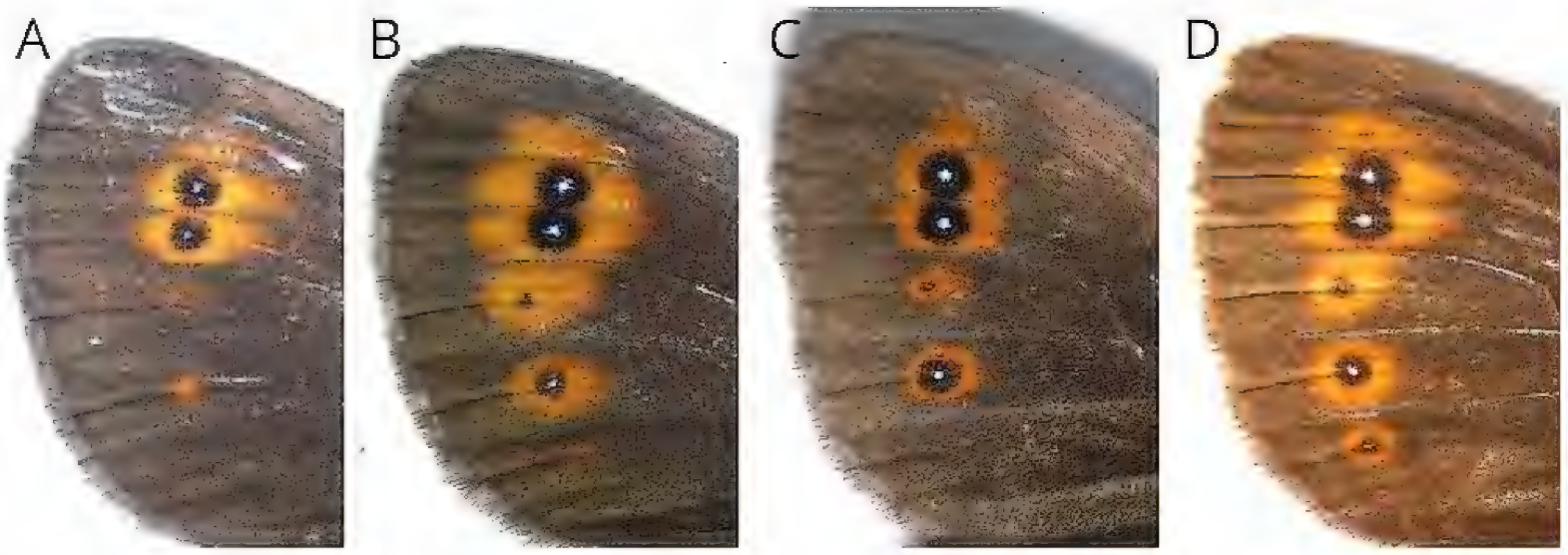

Figure 8. Wing pattern variability focused on the presence of eyespots with a white centre. **A.** 2, **B.** 3, **C.** 4, **D.** 5.

demonstrated on a bigger scale in the case of *Erebia euryale* (Esper, 1805) by Tatarinov and Kulakova (2013). These results may be explained by the association of temperature during development and the plastic response of wing pattern colouration, as shown by Taylor-Cox et al. (2020) in *Pararge aegeria* (Linnaeus, 1758). The function of a higher number of eyespots on a hidden surface of wings in *E. medusa* males from lower and warmer areas can be considered as a secondarily acquired, used especially in sexual selection (mate choice) or in predation deflection related to basking.

Variability of shape and size, as well as pattern and colouration of wings, may be related to dispersal, migration, territoriality, courtship and interactions with predators that vary across habitats. Moreover, temporal isolation and a shift in flight time were observed among localities with respect to temperature, even within geographically close localities separated by elevation.

Conclusions

To summarise our observations, trends in size along an elevational gradient were in line with a converse Bergmann's rule which states that size of body decreases with elevation. The wing shape variation showed that elongated wings, which allow for gliding flight to be maximised (Wootton 1992; Lentink et al. 2007; Kovac et al. 2012) were detected in higher, colder areas, while more rounded wings providing better manoeuvrability (Dudley 2002) were observed in lower, warmer areas. Our study showed that the wing size, especially of *E. medusa* butterflies, was likely influenced by temperature. Data indicates that spot reduction in cold locations is consistent with avoidance of detection by predators, but in warmer locations increased spotting may be associated with deflection of attacks to marginal wing areas (Prudic et al. 2015; Ho et al. 2016).

The specific flight behaviour of male butterflies and wing morphological traits may provide better insight into their morpho-functional role, but further research is needed for this purpose.

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Supplementary material 1

Supplementary File 1

Authors: Barbora Mikitová, Martina Šemeláková, Ľubomir Panigaj

Data type: JPG file.

Explanation note: Satellite view and elevation profile of the studied sites Kojšovská hoľa, (1246 m a. s. l) (A) and Zlatá Idka, (335 m a. s. l) (B).

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Link: https://doi.org/10.3897/nl.45.68624.suppl1

Supplementary material 2

Supplementary File 2

Authors: Barbora Mikitová, Martina Šemeláková, Ľubomir Panigaj

Data type: docx file.

Explanation note: Characterics of sampling sites: A) Kojšovská hoľa , B) Zlatá Idka, C) Dobroslava.

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Link: https://doi.org/10.3897/nl.45.68624.suppl2

Supplementary material 3

Supplementary File 3

Authors: Barbora Mikitová, Martina Šemeláková, Ľubomir Panigaj

Data type: docx file.

Explanation note: Average temperatures and occurrence of *Erebia medusa* according to sex during the flight period. KH – Kojšovská hoľa, ZL – Zlatá Idka, DB – Dobroslava.

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